SOME EFFECTS OF GROWTH SUBSTANCES ON LOW TEMPERATURE RESISTANCE AND QUALITY OF SUGAR BEETS

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The undersigned hereby certify that they have read and recommend to the School of Graduate Studies for acceptance, a thesis entitled "Some effects of growth substances on low temperature resistance and quality of sugar beets", submitted by S.R. Miller, B.Sc., in partial fulfilment of the requirements for the degree of Master of Science.

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THE UNIVERSITY OF ALBERTA

SOME EFFECTS OF GROWTH SUBSTANCES ON LOW TEMPERATURE RESISTANCE AND QUALITY OF SUGAR BEETS

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by

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INTRODUCTION

Reductions in yield and quality of crops, attributable to pre-harvest freezing temperatures, often occur in agricultural areas of this country. Regions with limited growing seasons are consequently adapted only for relatively hardy and early-maturing crops. Any means whereby a grower could lessen the hazards of frost injury would be of interest and potential importance.

The present study was undertaken to investigate the possible beneficial effects of applied plant growth regulators on the cold resistance and quality of the sugar beet (*Beta vulgaris*), which is an important specialty crop in southern Alberta and in other areas with favorable climate. Not infrequently, early fall frosts have undesirable effects on sugar beets in their regular areas of production. For example, Peto (27) has noted an immediate drop in sugar content of Alberta grown beets whose foliage was severely damaged by fall frost.

Interest in the possible practical value of applied growth regulating substances in extending the growing period of crop plants through protection against early frost, was motivated by results of a field experiment with parsnips at this university (7). Subsequent laboratory experiments under a limited range of controlled conditions with treated sugar beet and parsnip seedlings (8, 9, 10, 11) have given further encouragement for continued research along this line.

LITERATURE REVIEW

A number of investigators have been concerned with the effects of the application of growth substances to plants, and their subsequent effects on physiological activity, chemical composition, quality and yield.

For example, Brown (3) found that the total amount of water absorbed andtranspired by young bean plants (Phaseolus vulgaris) which had been treated with 2,4-dichlorophenoxyacetic acid,* was considerably less than for comparable untreated plants. The solid matter content of the above ground parts of the plant decreased rapidly after spraying with 1000 ppm. of the chemical, whereas increased accumúlation of solid matter was noted in the basal regions of the stems of plants sprayed with 25, 50 and 250 ppm. of 2,4-D. The treated beans had higher percentages of moisture, based on fresh weight of the intact plants, than did the untreated ones.

Rasmussen (29) reported that 2,4-D increased alcohol soluble nonprotein nitrogen two to five times in dandelions (Taraxacum officinale), whereas the protein nitrogen showed little variation between treated and untreated plants.

Working with bindweed (Convolvulus sepium), Smith and co-workers (33) found that the total nitrogen decreased in the leaves but increased in both stems and underground parts after 2,4-D treatment.

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* subsequently referred to as 2,4-D

Luecke, Hamner and Sell (22) recorded increased content of thiamine, riboflavin and nicotinic acid in leaves and stems of 2,4-D treated bean plants. Further work on beans (32, 36) showed an increase in the amino acids aspartic, lysine, valine, methionine and phenylalanine as well as increases in ash content, ether soluble fraction, unsaponifiable material and fatty acids following 2,4-D application.

Wheat (Triticum vulgare) treated with sodium trichloroacetate (TCA) in the greenhouse contained more protein and arginine in leaf tissue than untreated plants (30). There was also less ether extract, unsaponifiable material and fatty acids in the sprayed plants.

Analysis of samples, taken from the roots of dandelions of which the tops had been sprayed with 2000 ppm. of 2,4-D, indicated that reducing sugars increased rapidly for the first 15 days after which the amount decreased (29). The untreated plants contained a constant amount of reducing sugar throughout the experiment. Sucrose content as well as the polysaccharide fraction composed of dextrans and levulans decreased in the treated at a greater rate than in the untreated plants. Rasmussen concluded that the increase in reducing sugars was at the expense of the reserve carbohydrates.

Rebstock and co-workers (30) found that 30 pounds per acre of TCA applied to wheat seedlings grown in the greenhouse, increased the reducing sugars, acid hydrolyzable polysaccharides

and starch in leaf tissues. Root tissue analyses showed higher content of reducing sugars and starch although the acid hydrolyzable polysaccharides were decreased.

Smith, Hamner and Carlson (33) found increased reducing sugar content in leaves, stems and underground parts of bindweed sprayed with 2,4-D. Nelson (25) applied 2,4-D to sugar beets during mid-summer and found reduced sugar percentage without lowering of beet root yields whereas p-chlorophenoxyacetic acid reduced root yields but did not lower the sucrose percentage. Alpha naphthalene acetic acid did not give a favorable response in sugar content. Ririe et al.,(31) found that 2,4-D reduced significantly the percent sugar in treated sugar beets.

Several workers (28, 31) have shown that the sodium salt of 1,2-dihydropyridazine-3, 6-dione (maleic hydrazide) applied as a mid-summer foliage spray increased the percent sucrose in sugar beets with maximum effects after 21 days. Wittwer and Hansen (39) have reported as much as a 10 percent increase in sucrose due to sprays of maleic hydrazide. Peto and co-workers (28) found that maleic hydrazide applied in late September did not alter significantly the percent sugar nor yield of sugar beets. These workers (28, 39) found that storage losses were reduced by chemical treatment with maleic hydrazide. Peto (28) found sugar losses of maleic hydrazide treated beets were reduced up to 46 percent after exposure to freezing temperatures.

Stout (34) applied maleic hydrazide to sugar beets 13

days before harvest and found no significant differences between treatments in yield and sugar content. On storage, Stout found no significant difference in the respiration rate and sugar loss. These results were based on 2 years evaluation. Wittwer and Hansen (38) found that maleic hydrazide applied from 6 weeks to 48 hours before harvest did not influence yield, beet size or reduce the percent sucrose in beets at harvest time.

Numerous workers have used TCA and sodium 2,2-dichloropropionate (Dalapon) for control of weed populations in sugar beets (2, 6, 16, 26, 35, 37). These workers found that Dalapon could be applied at 3 pounds per acre or less as a pre-emergent spray with no effect on yield or sugar content of sugar beets. Williard and Ilnicki (37) and also Nelson (26) found TCA could be applied at rates up to 7.5 pounds per acre as a pre-emergent spray with no apparent effects on percent sugar, yield, or on beet counts either before thinning or at harvest time. Warren (35) found that at 6 pounds per acre TCA reduced top growth but by harvest time no differences were observed in sugar and beet yields.

Foliage application at rates as high as 7.5 pounds TCA per acre stunted sugar beets (37) whereas 5 pounds per acre did not when applied three weeks after planting. Dalapon at 2 to 3 pounds per acre after emergence of the sugar beets caused no visible injury to beets (37). Warren (35) concluded that Dalapon applied at rates higher than 4 pounds per acre as a foliage spray or repeated sprayings over 4 pounds reduced the yield significantly.

There is very little literature with direct reference to the effect of chemicals on frost resistance of plants. Fertilizer application, however, is known to have a bearing on maturation and hardiness of plants (5, 14, 23).

Levitt (21), after reviewing the effect of mineral nutrients on frost hardiness, concluded that excess nitrogen generally reduces hardiness whereas nitrogen deficiency increases it. In the case of fruit trees, nitrogen has been reported to increase hardiness. Levitt concluded that for most elements a deficiency reduces frost hardiness. Excess potassium has been reported to increase frost resistance (21).

Recently it has been shown that synthetic growth substances used in small amounts may have a beneficial effect on fruit development and cold hardiness of certain species.

For example, Crane (12) reports on the application of 2,4,5-trichlorophenoxyacetic acid (2,4,5-T) to Royal apricots 15 hours before the occurrence of a frost. At harvest time, over 3 weeks later, 76.2 and 12.3 percent of the fruit had dropped from the unsprayed and sprayed branches respectively. Of the fruits on the sprayed branches at the time of 2,4,5-T application, 66 percent reached maturity with normally developed embryos. The sprayed branches dropped 83.9 percent less fruit and also produced 69.7 percent more fruit with normally developed embryos than did the controls.

Crane postulates that the increased resistance to low temperatures may be attributable to an increase in sugar concentration in the cell sap since a pronounced increase in total sugar content, both sucrose and reducing sugars, has been noted after 2,4,5-T application to apricot trees.

With reference to vegetative parts of herbaceous species, Corns (7) has noted an improvement in frost resistance of parsnip foliage (Pastinaca sativa) sprayed in the field with one pound active per acre of 2,4,5-trichlorophenoxypropionic acid (Dow, Color Set) and sodium naphthaleneacetate (Dow, App-L-Set). Spray applications were made one month prior to the first frost. After the first below freezing temperatures the foliage of the chemically treated plants appeared to be unaffected whereas the untreated plants had foliage which was blackened. Apparently the chemicals had improved the hardening process in the treated parsnips. Later more severe frosts resulted in damage to the treated parsnips as well.

This finding led to laboratory experimentation under controlled conditions with beets, parsnips and other species of herbaceous plants. Some of this work has been discussed by Corns (8, 9) and Corns and Schwerdtfeger (11).

Corns (8), using etiolated sugar beet seedlings grown in petri dishes containing vermiculite and solutions of 0, 4 and 8 ppm. of Dalapon found increased resistance to low temperature exposure in the treated seedlings. In these laboratory experiments a

temperature of -10 degrees Centigrade for intervals of 5 or 6 minutes was used and the number of surviving plants were recorded. Differences in survival between treated and untreated material were as high as 35 percent with the treated seedlings always showing a greater percentage survival than the untreated seedlings. Two freezing techniques were used, one involving the whole seedlings in the vermiculite and the other, shoots severed at their base and attached to strips of scotch tape. This latter method was used in later experiments.

Corns and Schwerdtfeger (11), in a later communication, using a similar technique, reported increased resistance to low temperatures in sugar beet as well as garden beet seedlings when treated with either Dalapon or TCA. These authors were unable to demonstrate improvement in cold resistance of Altaswede Red clover, Ferax alfalfa and Pencil Pod Wax beans treated in a similar manner. In further trials at the same university promising results have since been obtained with parsnips treated with Dalapon, Color Set and App-L-Set (10).

In a later publication (9), reporting more extensive work with various chemicals and formulations, Corns found a similar increased resistance in sugar beet seedlings treated with the sodium salts, sodium 2,2-dichloropropionate, sodium trichloroacetate and sodium-trichloropropionate as well as the free acids 2,2-dichloropropionic and trichloroacetic. These substances all have a similar structure. In all cases concentrations of 4 and 8 ppm. were significantly superior to the untreated

controls with regard to frost resistance. No differences, however, were observed with isopropyl n-phenyl carbamate (IPC), 3-amino-1,2, 4-triazole (AT) nor with sodium chloride at 0, 4 and 8 ppm.

In the same series of tests, hardening studies of seedlings kept at 6 degrees Centigrade in a weakly illuminated chamber indicated significant positive response in Dalapon treated seedlings although greater variability was observed than with the etiolated seedlings. The cold hardening treatments tended to increase the magnitude of difference between Dalapon treated and control plants.

EXPERIMENTAL

A. Field Experiments

1. Objectives

Field experiments were conducted in both 1954 and 1955 to determine the effects of the growth substances Dalapon and TCA, applied at various rates and stages of development, on low temperature resistance and quality of sugar beets. The literature reviewed previously suggested that by manipulating the rates of application of the growth substances at various stages of growth the resistance to low temperatures might be improved.

In the same experiments the effects of the chemicals on percent sugar, percent nitrogen, percent dry matter, sugar yield per acre as well as yield of storage roots were to be studied as an

assessment of root quality and crop returns. There was also the possibility of being able to correlate certain of these fractions with increased low temperature resistance, if found.

During the 1955 field experiment determination of the desiccation rate of leaves, removed from plants that had received various chemical applications were thought to be instructive. Earlier laboratory trials indicated that variations in water retention by variously treated plants were of some significance in understanding the action of the chemicals.

2. Materials and Methods of Treatment

1954 Field Test

On May 21 intact sugar beet seed balls of the variety Kuhn-TW-3 were planted in a field of Black Lacustrian soil at Edmonton. A Columbia seeder was used for planting the single row plots 22 feet long with 3 feet between rows. The field plan consisted of randomized chemical treatments within each of the 4 replicates.

After the beets had emerged and were at the 2 to 4 leaf stage of growth they were thinned to obtain 6 to 9 inch spacings between individual plants. Throughout the summer months weeds were removed in order to maintain uniformity in the experimental plots.

Chemical spray application consisted of two chemicals, Dalapon and TCA applied at various rates and stages of growth to

sugar beet foliage. The stages of growth and rates of application were: Stage 1 - Early Seedling - July 14, Stage 2 - Early Foliage - July 30, Stage 3 - Mid-Season Foliage - August 30, Stage 4 - Late Foliage - September 15, and Stage 5 - Repeated Application - July 30, August 30 and September 15.

The rates used were as follows: At stages 1, 2, 3 and 4 Dalapon was applied at 1 and 2 pounds active chemical per acre and TCA at 2 and 4 pounds active ingredient per acre. The repeated application, designated as stage 5 involved rates of 0.25 and 0.50 pounds per acre of Dalapon or TCA. In all cases reported in this thesis rate 1 was the lower and rate 2 the higher rate of application. The amounts of chemical refer to the active ingredient in the various products used.

The chemicals were applied in a volume of 70 gallons of solution per acre in order to obtain uniform coverage of leaf surface. The spray was applied with a mobile power paint sprayer, operated at a pressure of 30 pounds per square inch, fitted with a modified sprayer head and fan spray nozzle.

1955 Field Test

Decorticated sugar beet seed of the variety Kuhn-TW-3 was planted on May 16 in the same field area used for the 1954 test. Each single row plot was 25 feet in length with 3 feet between rows. The treatments were again randomized within each of the 4 replicates.

As in 1954 the plots were thinned to obtain 6 to 9 inch spacings, and weeded throughout the summer. In addition, the plots

were irrigated periodically during the months of July and August owing to the prevalent dry weather.

The two chemicals Dalapon and TCA were applied at various rates and stages of beet development. The stages of growth and dates of application were: Stage 1 - Pre-emergent - May 17, Stage 2 - Early Foliage - July 13, and Stage 3 - Repeated Application - July 13 and August 20.

Dalapon as a pre-emergent spray was applied at rates of 2, 4 and 8 pounds per acre and TCA at 4 and 8 pounds per acre. Foliage applications consisted of 250 ppm. (0.16 pounds per acre) and 500 ppm. (0.32 pounds per acre) of Dalapon or TCA. Repeated foliage application consisted of 50 and 100 ppm. or 0.032 and 0.064 pounds per acre respectively of each of the 2 chemicals. As in 1954, the equivalent to 70 gallons of water per acre was used in all of the sprayings with a small amount of Dreft (.05 grams) added to give uniform wetting.

3. Methods of Observation and Analyses

Leaf Material

The progressive effect of the various chemical treatments on sugar beet foliage was determined by visual observation in both 1954 and 1955. After the first below freezing temperatures notes were taken to determine the effect of low temperature exposure on survival of leaf material.

On August 11, 1955, leaf samples were taken for measurements

of the rate of fresh weight lost from the treated and non-treated sugar beets. Four uniform leaves approximately 12 square inches in area, which were too young to have received the chemical application, were taken from the central portion of the crown, placed in beakers of water and taken immediately to the laboratory. The rate of weight lost was determined by the method of Mitchell and Marth (24) which consisted of drying the leaf samples in static air. The amount of weight lost was determined after intervals of 1, 2, 4 and 24 hours and was designated as water loss.

Root Material

After the leaf material was frozen the sugar beets were dug, topped and as much dirt as possible was removed by scraping. Twenty of the most uniform beets were selected from each single row plot, placed in open mesh onion bags and stored in the root cellar at 40 degrees Fahrenheit until the individual beets were sampled.

(a) Method of Sampling Sugar Beet Roots in 1954

The sugar beet roots were removed from the root cellar and washed with the aid of a scrub brush. Individual root samples from 20 beets were then obtained, as cores, by means of a one-half inch cork borer inserted at an angle of approximately 45 degrees for $1\frac{1}{2}$ inches into the shoulder of the beet. The core thus obtained extended to the central axis of the root. Subsequently the beet root plugs were finely ground in a meat grinder fitted with burr openings. Duplicate 26 gram samples of this fresh material were immediately weighed into labelled polyethylene bags which were sealed and placed

in a freezer chest at -20 degrees Centigrade until the biochemical determinations were made. A further sample was weighed directly into weighed aluminum drying cans for determination of dry matter and nitrogen.

(b) Method of Sampling Sugar Beet Roots in 1955

After digging and topping the sugar beets, the 20 most uniform storage roots were selected from each plot, placed in onion bags and weighed to the nearest .25 pound before being placed in the root cellar at 40 degrees Fahrenheit.

Samples from each root were obtained, after washing, by cutting the beet vertically into quarters. A $\frac{1}{4}$ inch thick section, extending the full length of the root was obtained from the surface of one of the quarters. After each of the 20 roots were sampled the sections were ground in a hammer mill. The resulting pulp was thoroughly mixed and duplicate 26 gram samples were weighed directly into polyethylene bags, sealed and placed in storage at -20 degrees Centigrade. Additional samples of approximately 30 grams were weighed into aluminum drying cans to be used for determinations of dry matter and nitrogen content.

(c) Methods of Biochemical Analyses of Root Material

Digestion of Root Pulp

Ground pulp, as obtained by the various methods outlined earlier, was washed into 200 ml. Kohlrausch flasks with approximately 180 ml. of distilled water. The samples in the flasks were

allowed to warm to room temperature before being placed in a constant temperature water bath at 70 - 75 degrees Centigrade for 40 minutes. After the digestion period the flasks were cooled in running water and the cooled contents made up to volume. This method is similar to the one being used at the Taber Sugar Beet Factory.

Polarimetric Analyses

A 50 ml. portion of the extract from the digestion process was placed in a test tube, clarified with lead subacetate (basic dry powder for sugar analysis) and filtered. The clarified solutions were transferred to 200 mm. polarimeter tubes and polarized in a Pellin polarimeter. The readings obtained were converted to percentage sucrose.

Refractometric Analyses

A portion of the uncleared extract was transferred to a Zeiss immersion refractometer. Readings obtained on the refractometer were converted to percent soluble solids by means of tables in Brown and Zerban (4).

Determination of Dry Matter

The ground pulp was transferred directly to weighed aluminum dry cans. The samples were dried at 55 degrees Centigrade in a forced draft oven until constant weight was obtained. The dry weight of the sample was used in calculating the percentage of dry matter.

Determination of Nitrogen

Samples which had been used for moisture determinations were ground in a Wiley mill equipped with a 20 mesh screen. After grinding, the samples were dried at 100 degrees Centigrade until their weight became constant. Approximately 1 gram samples were weighed into Kjeldahl flasks and nitrogen determined by the official method (1).

Determination of Total and Reducing Sugars

A 5 ml. aliquot of the filtered digestion mixture was transferred to a 50 ml. beaker. After precipitation with neutral lead acetate, the excess lead was removed with a saturated solution of disodium phosphate, before filtering on a Buchner funnel. The cleared solution was made up to volume and reducing sugar determined by the method of Hassid (18, 19). A portion of the cleared extract was hydrolyzed with hydrochloric acid and total sugars determined by Hassid's method with the results expressed as glucose.

Determination of Root Yield

Yields were obtained by weighing 20 of the most uniform storage roots obtained from the 25 foot plots. This weight representing 75 square feet was multiplied by a factor to obtain the yield per acre. Other yields were obtained by multiplying the root yield per acre by the percentage of each fraction.

Determination of Purity

Purity was obtained by dividing the percent sucrose obtained by the polarimeter by the percent solids from the refractometer reading. This was multiplied by 100 to give percentage.

4. Results of Field Experiments

A. Effects of Chemical Treatments on Above Ground Parts of Sugar Beets

(a) Effect of Chemicals on Foliage (1954 and 1955)

On September 15, 1954, severe leaf burning was noted in all chemically treated plots except the plots receiving the late foliage application. Leaf burning consisted of chlorosis around leaf margins, especially in the outer leaves to which the chemicals were applied. The new leaves which apparently had arisen since the application appeared to be unaffected.

On July 18, 1955, 9 weeks after planting the second year's plots, the pre-emergent sprays of both Dalapon and TCA appeared to have stunted the sugar beets. At the 8 pounds per acre level some leaf burning and chlorosis was observed in both Dalapon and TCA treated plots. By August 20 no differences could be observed between the pre-emergent applications and the untreated controls. The post-emergent applications did not have any visible effects on the sugar beets.

(b) Effect of Chemical Treatments on Low Temperature Resistance (1954 and 1955)

During the night of September 17, 1954, the air temperature dropped to 25 degrees Fahrenheit with no apparent effect on sugar beet foliage although potato and tomato tops were blackened and "water soaked". More severe temperatures of 20 degrees Fahrenheit occurred on October 4 which resulted in very severe damage to the foliage of the sugar beets. There were no visible differences in resistance to low temperature exposure attributable to any chemical treatment.

Roots from three replicates of the 1954 sugar beet plots were dug, topped and put in storage on October 5 and 6. One of the replicates was not included because a portion of it remained under water for a large part of the summer.

On September 9, 1955, the temperature dropped from 60 degrees Fahrenheit during the afternoon to 18 degrees during the night. This low temperature resulted in severe foliage damage to both treated and untreated beets. No visible differences were detectable between sprayed and unsprayed plots. On account of the early frost, applications of the chemicals during the late foliage stage were not made.

The 1955 sugar beet plots were dug on September 19 and 20 and stored in the root cellar for subsequent analyses.

(c) Effect of Chemical Applications on Rate of Water Loss

Table 1 gives the mean percentage of the amount of water lost as determined by the method of Mitchell and Marth (24) after 1, 2, 4 and 24 hour periods of desiccation at 25 degrees Centigrade. The samples were taken on August 11, 1955, 12 weeks after planting.

Although no significant differences were found between the various treatments several trends are indicated. Dalapon applied as a foliage spray at the lower rate in each stage tended to increase the amount of water lost to a level above that of the controls, whereas the loss from leaves of plants treated at the higher rate appears to have been comparable to that of the controls. With TCA, on the other hand, the plants receiving the lower rates of application in each stage, lost an amount of water approximately the same as for the controls whereas the higher rates of application seemed to cause a greater loss of water. It should be pointed out, however, that there was considerable variability between replicates even though the differences between replicates were not significant after the first hour of desiccation.

Table 1

Percentage of Fresh Weight Lost from Detached Leaves

of Sugar Beet Plants Sprayed with Growth Substances

Treatment No.	Percent Loss in Weight Following Various Desiccation Periods After Sampling August 11, 1955 1 Hour	2 Hours	4 Hours	24 Hours
** 0	*** 11.6	16.0	21.0	49.4
1	11.2	17.0	24.0	56.6
2	13.0	17.8	24.2	60.0
3	11.8	17.6	25.2	62.9
4	14.7	22.4	32.0	67.0
5	12.3	18.0	26.5	72.5
6	14.7	20.9	27.3	57.0
7	11.6	16.6	22.7	55.5
8	11.6	16.4	22.1	52.4
9	17.6	24.7	31.6	65.2
10	15.0	20.6	26.2	55.6
11	11.9	17.5	23.8	53.6
12	14.0	18.5	23.7	50.4
13	14.2	20.6	27.4	59.6

Mean Square Values from Analyses of Variance

Replicates	38.393*	65.743	75.843	51.100
Treatments	13.725	26.050	42.321	175.123
Error	12.872	31.592	52.639	92.324

* Significant at 5 percent level
** See legend on page 23a
*** Mean of 4 replicates

B. Effects of Chemical Treatments on Storage Roots of Sugar Beets

Table 2 gives the mean percentage of the various components of the sugar beet roots for which analyses were made during 1954. The results show no significant differences within the fractions between the various treatments. It should be noted that differences between replicates were very pronounced.

More extensive analyses of the effects of chemical treatments on the constituents of sugar beet roots are given in Table 3 for the 1955 material. The data show the means of the 4 replicates for the various fractions in the samples from the various treatments. It appears that Dalapon and TCA applied at 8 pounds per acre as a pre-emergent spray reduced the root yield and yield of sucrose per acre by a significant amount with TCA showing the greatest amount of reduction. At 4 pounds per acre, applied to the soil, both chemicals lowered the percent sucrose and yield of sucrose with TCA again showing the greatest effect. The sucrose percentage in roots treated with 4 pounds TCA per acre was significantly lower at the 5 percent level than it was for the corresponding checks. It is evident that TCA as a pre-emergent spray applied at 4 and 8 pounds per acre increased significantly the percentage nitrogen.

Foliage sprays at stages 2 and 3 did not have statistically significant effects on the various fractions even though certain trends are suggested by the respective data.

Table 2

Analyses of Sugar Beet Roots from Field Experiment (1954)++

Treatment Number	Percent				
	Sucrose	Solids	Purity	Dry Matter	Nitrogen
+ 0	14.28	18.30	75.40	21.22	1.06
0	14.50	18.28	79.28	21.43	0.96
1	14.51	19.00	76.40	21.69	1.06
2	14.88	19.26	77.43	21.38	1.10
3	13.48	18.72	71.98	22.06	0.96
4	11.66	18.89	61.73	21.02	1.03
5	13.59	18.95	71.65	21.67	1.01
6	13.50	17.90	75.39	20.87	1.02
7	14.84	19.10	77.64	22.00	1.02
8	13.32	18.44	77.62	21.52	1.10
9	14.56	19.15	76.12	21.34	1.03
10	13.42	18.13	74.03	21.17	1.10
11	13.89	18.51	75.03	21.03	1.10
12	14.23	17.28	82.33	20.90	1.11
13	14.21	18.80	75.66	21.28	1.01
14	14.04	18.19	77.18	21.30	1.10
15	14.58	19.30	76.00	21.47	1.02
16	14.11	18.25	77.58	21.24	1.07
17	14.97	19.15	78.40	22.48	0.96
18	13.63	17.38	79.21	20.80	1.03
19	14.34	18.69	76.77	22.09	1.03
20	14.70	18.90	77.89	21.42	0.96

Mean Square Values from Analysis of Variance

Replicates	7.515**	3.11	110.1	12.595**	0.035*
Treatments	1.555	0.97	43.75	0.561	0.0078
Error	0.969	1.06	37.43	0.560	0.0069

\+ See legend on page 23a
++ Each figure mean of 3 replicates
* Significant at 5% level
** Significant at 1% level

Table 3

Analyses of Sugar Beet Roots from Field Experiment (1955)++

Treatment Number	Percent Reducing Sugars	Percent Sucrose	Percent Total Sugars	Tons/Acre Storage Roots	Pounds per Acre Total Sugars	Pounds per Acre Sucrose	Percent Dry Matter	Percent Solids	Percent Purity	Percent Nitrogen
+ 0	0.58	15.80	18.83	7.369	2768	2322	23.74	18.82	83.89	1.04
1	0.48	15.35	18.40	7.260	2668	2233	22.86	19.52	78.69	1.06
2	0.52	14.94	18.11	7.440	2668	2204	22.43*	18.82	79.44	1.12
3	0.60	14.83	18.17	5.844*	2142	1742*	21.90**	18.72	79.22	1.10
4	0.45	14.44*	16.94	6.861	2325	1987	21.57**	17.62	81.31	1.18*
5	0.54	15.30	18.72	5.590*	2100	1708**	23.69	18.88	81.37	1.13*
6	0.60	15.91	18.91	7.691	2802	2447	23.78	19.44	81.71	1.04
7	0.56	15.62	18.35	7.922	2910	2470	23.60	19.51	82.09	1.11
8	0.54	16.01	17.88	7.441	2675	2392	23.46	19.34	82.82	1.05
9	0.55	15.94	17.76	7.768	2772	2475	23.20	18.82	84.59	1.03
10	0.56	16.49	19.54	7.369	2878	2432	24.26	19.95	83.71	1.04
11	0.58	15.85	18.58	7.804	2898	2473	23.30	19.13	83.12	1.02
12	0.55	15.66	18.46	7.623	2808	2386	22.78	18.95	82.70	1.12
13	0.66	14.83	18.96	8.276	2872	2549	23.14	18.86	81.94	1.08
L.S.D. at 5% level	--	1.06	--	1.478	--	460	1.30	--	--	0.09
L.S.D. at 1% level	--	--	--	--	--	614	1.74	--	--	--

Mean Square Values from Analysis of Variance

Replicates	0.205**	0.106	7.233**	7.672**	800,133	769,560**	2.463*	7.830**	121.4**	0.0087
Treatments	0.011	1.161*	1.586	2.248*	298,915	298,469**	2.268**	1.171	12.83	0.0085*
Error	0.013	0.550	1.608	1.072	218,262	103,691	0.830	0.694	11.67	0.004

\+ See legend on page 23a

++ Each figure mean of 4 replicates

* Significant at the 5% level

** Significant at the 1% level

Legend for Tables 1, 2 and 3

Legend for Tables 1 and 3

Treatment Number	Chemical, Rate and Date of Application
0	Control, untreated
1	Dalapon, 2#/acre, May 17
2	Dalapon, 4#/acre, May 17
3	Dalapon, 8#/acre, May 17
4	TCA, 4#/acre, May 17
5	TCA, 8#/acre, May 17
6	Dalapon, 0.16#/acre, July 13
7	Dalapon, 0.32#/acre, July 13
8	TCA, 0.16#/acre, July 13
9	TCA, 0.32#/acre, "
10	Dalapon, 0.032#/acre, July 13, August 20
11	Dalapon, 0.064#/acre, July 13, August 20
12	TCA, 0.032#/acre, July 13, August 20
13	TCA, 0.064#/acre, July 13, August 20

Legend for Table 2

Treatment Number	Chemical, Rate and Date of Application
0	Control, untreated
0	Control, untreated
1	Dalapon, 1#/acre, July 14
2	Dalapon, 2#/acre, "
3	TCA, 2#/acre, "
4	TCA, 4#/acre, "
5	Dalapon, 1#/acre, July 30
6	Dalapon, 2#/acre, "
7	TCA, 2#/acre, "
8	TCA, 4#/acre, "
9	Dalapon, 1#/acre, August 30
10	Dalapon, 2#/acre, "
11	TCA, 2#/acre, "
12	TCA, 4#/acre, "
13	Dalapon, 1#/acre, September 15
14	Dalapon, 2#/acre, "
15	TCA, 2#/acre, "
16	TCA, 4#/acre, "
17	Dalapon, 0.25#/acre, July 30, August 30, September 15
18	Dalapon, 0.50#/acre, July 30, August 30, September 15
19	TCA, 0.25#/acre, July 30, August 30, September 15
20	TCA, 0.50#/acre, July 30, August 30, September 15

5. Discussion and Conclusions from the Field Experiments

The rate of fresh weight loss in the determinations on detached leaves indicated, as noted earlier, that the chemical applications of TCA and Dalapon to sugar beet foliage did have an effect, although the differences were not statistically significant. Mitchell and Marth (24) have reported a decreased rate of water loss in severed bean plants dipped in various growth substances. In none of the literature reviewed have workers reported an increased rate of water loss after applications of TCA and Dalapon. An increase as noted in the present work, may, if real, be attributed to the relatively low concentrations of chemicals used in this field experiment as compared with higher concentrations used in the other experiments reported in the literature. It is possible that if a more uniform method of leaf sampling were used with greater replication, the trends which are indicated would be established more firmly. It would be of interest to undertake more intensive studies of this nature including possible correlation with yield and quality of the plants as manipulated by applied chemicals.

With regard to the effects of chemicals on frost resistance, it has been pointed out that in the field experiments involving Dalapon and TCA at various rates applied at different stages of plant development during 1954 and 1955 there were no visible differences attributable to the chemicals used. Some of the positive laboratory results in more closely controlled

experiments related to this phase of the investigation are recorded later.

With reference to the influence of applied chemicals on sucrose content of sugar beets, the present results are similar in certain respects to those reported by Warren (35) as well as by Williard and Ilnicki (37). Warren found that Dalapon applied at 4 pounds per acre or less as a foliage spray did not reduce yield of sucrose or percent sucrose. Similarly, Williard and Ilnicki found that 5 pounds per acre of TCA as a foliage spray did not affect these same constituents.

Analysis of roots from plots treated prior to plant emergence during the 1955 experiment indicated that TCA at 8 pounds per acre adversely affected the sugar beet root yields and total sucrose per acre. Williard and Ilnicki (37) and Nelson (26) found that 7.5 pounds per acre did not affect yield or percent sucrose. From the experimental data given here it can be seen that 4 pounds of TCA reduced the percent sucrose significantly and tended to lower the yield of sucrose and total sugars when compared to the controls.

Berrett (2) found that Dalapon at 3 pounds per acre as a pre-emergent spray did not affect the yield of sugar beets. Williard and Ilnicki (37) have stated that pre-emergent sprays of Dalapon up to $4\frac{1}{2}$ pounds per acre did not adversely affect sugar beets. From the field results recorded in the present work it would appear that Dalapon at 2 and 4 pounds per acre applied before emergence did

lower the total yield of sucrose as well as the percent sucrose under the conditions at Edmonton, although the amount was not significantly different from the untreated checks.

Although all pre-emergent sprays resulted in a lowering of percent and yield of sucrose as compared to the checks, the nitrogen content was higher in all treated material as compared with the untreated controls. It appears that when the percent sucrose was lowered to a level below that of the checks the percent nitrogen was higher than the checks.

The foliage applications of Dalapon and of TCA during 1955 had no statistically significant effect on the various fractions analyzed. However, several trends are indicated. For example, the percent sucrose in the samples within the various stages appears to be higher as a result of the lower concentration of each chemical. The same trend is found on analyzing the percent total sugars, percent dry matter and percent solids. In contrast, the higher root yields, total yield of sugar and yield of sucrose appear to be associated with the higher rate of application of each chemical in each stage. In all cases the foliage applications tended to increase the amount of sucrose present per acre when compared to the unsprayed checks. The increase in yield of sucrose varied from 64 pounds to 227 pounds per acre. The percent increase over the untreated checks varied from 3 to 10 percent. This finding of an increase in yield of sucrose after chemical application would present an interesting potentiality, if the findings were substantiated in further large-scale tests.

B. Laboratory Experiments

1. Objectives

Laboratory experiments were undertaken to study the effects of the growth substances Dalapon and TCA on etiolated seedlings. Corns (8, 9, 11), as reported earlier, found an increased resistance to low temperature exposure in seedlings treated with these two chemicals. In addition it was thought to be useful to measure the injury of the seedlings by determining the amount of exosmosis from the seedlings following low temperature exposure.

Biochemical analysis of the etiolated sugar beet seedlings was undertaken to determine possible differences between the treated and non-treated seedlings. The fractions to be studied included moisture, content, percent reducing sugars, percent total nitrogen and percent water soluble nitrogen. The literature reviewed suggested that some of the above fractions respond quite noticeably to application of growth substances. These fractions might then be correlated with increased resistance to low temperature exposure.

Further experiments were undertaken to measure the rate of desiccation of the seedlings since this is another factor which has been reported to be correlated with frost resistance (21).

2. Materials and Methods of Treatment

Decorticated sugar beet seed balls of the variety Kuhn-TW-3 were planted in flat-bottomed bacterial culture petri dishes.

The procedure followed was similar for all the experiments and consisted of spreading 50 ml. of aggregate grind vermiculite (acid magnesium silicate) as a uniform layer within the petri dish. On the surface of this layer 65 sugar beet balls were distributed before the addition of a covering layer of 50 ml. of vermiculite. To each dish 65 ml. of distilled water or solutions of 4, 8 or 12 ppm. of Dalapon or TCA were added. Thus, the amount of active chemical added to each dish was 0, 0.0026, 0.0052 or 0.0078 grams respectively of Dalapon or of TCA.

Following the addition of the various solutions, the petri dishes were placed in the dark on trays in a germinating cabinet maintained at a constant temperature of 20 degrees Centigrade and maximum humidity. The dishes containing the seedlings were removed from the cabinet after a 7 day growing period and the moisture adhering to the seedlings was allowed to evaporate before samples were removed from each dish for the various experiments conducted.

3. Methods of Analyses

Method of Freezing Sugar Beet Shoots

The method used was introduced by Corns (8). In this technique 25 uniform etiolated sugar beet seedlings were severed at their shoot-base and attached to $\frac{1}{2}$ inch bands of scotch tape suspended across wire frames. The severed shoots were attached in such a manner that their upper region extended at least 1 inch beyond the forward edge of the tape. The frames, supporting the

bands of plant material, were placed, one replicate at a time, for a period of 5 minutes on a slowly revolving tray in a cold chamber operated at a temperature of -10 degrees Centigrade. At the end of this time interval the frames were removed from the chamber and allowed to thaw at room temperature. Following a thawing period of 5 to 10 minutes the number of surviving, turgid, healthy shoots were counted. The stems considered to be killed were those that had collapsed and had become limp, translucent and "water soaked". The percent of the seedlings killed by the low temperature exposure was calculated from the ratio of the number killed to the total number of seedlings attached to the scotch tape.

Determination of Specific Conductance of Sugar Beet Seedlings Exposed to Low Temperatures

The method outlined by Dexter and co-workers (13, 15) was modified to accomodate the small size of sample used here. The procedure consisted of weighing 1 gram samples, each containing approximately 25 seedlings which were removed intact from the vermiculite, into 50 ml. beakers. Each replicate, which consisted of 4 beakers, one for each rate of application, was placed on the revolving tray in the cold chamber for a period of 5 minutes at a temperature of -10 degrees Centigrade. After removal from the chamber the beakers and contents were allowed to warm to room temperature before 25 ml. of distilled water was added to each. The beakers were then covered with a layer of parafilm and held for 24 hours in a constant temperature water bath at a temperature

of 25 degrees Centigrade. At the end of this period the specific conductance of the solutions was measured on a Solu-Bridge Soil Tester.

The total extractable electrolytes present were determined on similar samples. In this technique the intact sugar beet seedlings were placed in 50 ml. beakers to which 25 ml. of distilled water had been added. The beakers and contents were then placed in an autoclave operated at a pressure of 15 pounds per square inch for a period of 5 minutes. Following the removal and subsequent cooling, the beakers were covered with parafilm before being placed in the water bath at 25 degrees Centigrade for 24 hours. The total electrolytes present were determined by measuring the specific conductance of the extracts.

The injury to the seedlings caused by the exposure to low temperatures was expressed as a percentage index based on the ratio of the specific conductance after low temperature exposure to the specific conductance after autoclaving.

Determination of the Rate of Fresh Weight Lost from Sugar Beet Seedlings

From each petri dish, 25 uniform etiolated sugar beet seedlings were selected, severed at their base and placed in 50 ml. beakers. The initial fresh weight was recorded. Later, after intervals of 1, 2, 6 and 24 hours exposure of the material to room temperature in static air, the changes in weight were determined. The amount of weight lost was used as an index of the percent water

lost after each time interval on the basis of the original fresh weight. The method of drying at room temperature was similar to that used by Mitchell and Marth (24).

Determination of Reducing and Total Sugars

Fifty intact etiolated seedlings from each treatment were removed from the vermiculite and weighed directly into duplicate extraction thimbles. A Soxhlet apparatus was used for a 6 hour extraction with 80 percent ethanol. Following extraction the samples were evaporated before being cleared with saturated neutral lead acetate and saturated disodium phosphate. The samples were filtered on a Buckner funnel before reducing sugars were determined by the method of Hassid (18, 19). An aliquot of the cleared extract was hydrolyzed with hydrochloric acid and neutralized, after 24 hours, with sodium carbonate. This hydrolyzed sample was used for the determination of total sugars by Hassid's method. Results were expressed as glucose.

Determination of Dry Matter and Moisture Content

Approximately 100 etiolated sugar beet seedlings were removed intact from the vermiculite and transferred into weighed aluminum drying cans. The containers and samples, after weighing, were dried at 100 degrees Centigrade until a constant weight was reached. The loss in weight was used to calculate the percent moisture. The percent dry matter was determined by difference.

Determination of Total Nitrogen

The total nitrogen of the sugar beet seedlings was determined on the same sample as was used for moisture content. After drying the samples were ground with a mortar and pestle and redried at 100 degrees until constant weight was again obtained. Duplicate samples of 10 to 35 mgm. were weighed on cigarette papers and transferred to microkjeldahl flasks. The official microkjeldahl method (1) was used with a 4 hour digestion period.

Determination of Water Soluble Nitrogen

The samples used for determination of water soluble nitrogen were obtained from the bulk samples for which the moisture content is given. Duplicate 50 mgm. samples were weighed into 50 ml. erlenmeyer flasks to which 20 ml. of water had been added. The samples were shaken for 16 hours to extract the water soluble fraction. Following filtration an aliquot of the filtrate was placed in a microkjeldahl flask containing 2 ml. of the Galston and Dalberg digestion mixture (17). Superoxol was added to the digestion mixture as described in their method. The distillation was carried out using the official microkjeldahl method for total nitrogen (1).

4. Results of Laboratory Experiments

In Table 4 are given the results of the root and shoot measurements of the Dalapon and TCA treated seedlings.

Table 4

Root and Shoot Measurements of the 25 Longest Etiolated Sugar Beet Seedlings after a 7 Day Growing Period in Various Chemical Solutions

A. Dalapon Treated Seedlings

Treatment	** Length in cm. Roots	Shoots
Check	7.113	6.688
4 ppm.	6.825	6.435
8 ppm.	6.669	6.281
12 ppm.	6.220*	6.151
L.S.D. at 5% level	0.537	---

B. TCA Treated Seedlings

Treatment	** Length in cm. Roots	Shoots
Check	6.856	6.078
4 ppm.	6.688	6.188
8 ppm.	6.611	6.027
12 ppm.	6.794	5.788*
L.S.D. at 5% level	---	0.258

* Significant at 5 percent level ** Mean of 4 determinations

Dalapon at 12 ppm. inhibited root development significantly at the 5 percent level whereas TCA at 12 ppm. inhibited shoot development at the same level. Although the differences were not significant, Dalapon at 4 and 8 ppm. tended to reduce root and shoot measurements to a level below that of the checks. Results with TCA were not as conclusive.

Table 5 provides a summary of the results obtained after exposing etiolated sugar beet seedlings, treated with 0, 4, 8 and 12 ppm. of Dalapon and TCA, to low temperatures. The amount of injury to the seedlings was measured by 2 methods, viz. (a) freezing the seedlings on bands of scotch tape and making survival counts, and (b) determining the extent of injury based on the amount of exosmosis of electrolytes following low temperature exposure.

Table 5

Percent Injury to Sugar Beet Seedlings Exposed to -10 Degrees Centigrade for a Period of 5 Minutes as Measured by Different Methods

A. Dalapon Treated Seedlings

Treatment	* Freezing and Visual Observation (Tape)	** Percent Injury on Bases of Conductivity Ratio
Check	54	73
4 ppm.	35	63
8 ppm.	29	52
12 ppm.	28	45

B. TCA Treated Seedlings

Treatment	* Freezing and Visual Observation (Tape)	** Percent Injury on Bases of Conductivity Ratio
Check	58	71
4 ppm.	39	50
8 ppm.	35	57
12 ppm.	37	53

* Mean of 10 individual determinations
** Mean of 4 individual determinations

The data for conductivity ratio have certain limitations which will be discussed later. It can be seen from the table that treatment of the sugar beets with Dalapon and TCA prior to low temperature exposure resulted in plants being more resistant than the corresponding untreated controls as judged by either method of determining injury. The treated seedlings showed as much as 28 percent less injury than the corresponding untreated controls.

Table 6 gives typical results obtained by measuring the loss in weight from detached etiolated sugar beet seedlings which had received treatment with Dalapon and TCA. The seedlings treated with Dalapon at concentrations of 8 and 12 ppm. lost amounts of weight significantly higher, at the 1 percent level, than the corresponding untreated controls after a time interval of 1 hour or more. It is interesting to note that seedlings treated with 4 ppm. of Dalapon lost a greater amount of weight, significant at the 5 percent level, only after 2 hours or more of desiccation when compared to the checks. That is to say, there was, with increasing concentration of Dalapon an increasing amount of weight lost from the detached sugar beet seedlings. Presumably this loss in weight was mainly due to water loss.

Table 6

Percentage of Fresh Weight Lost from Sugar Beet Seedlings After Various Periods of Desiccation at Room Temperature

A. Dalapon Treated Seedlings

Treatment	Percent Loss in Weight Following Various Desiccation Periods			
	1 Hour	2 Hours	5 Hours	24 Hours
Check	+16.80	29.66	60.09	85.11
4 ppm.	17.79	33.46*	66.34*	88.37*
8 ppm.	19.27**	34.47**	69.58**	89.68**
12 ppm.	20.41**	36.08**	71.58**	90.17**
L.S.D. at 5% level	1.59	3.01	6.01	3.10
L.S.D. at 1% level	2.29	4.32	8.64	4.46

+ Mean of 4 replicates desiccated at 25 degrees Centigrade

B. TCA Treated Seedlings

Treatment	Percent Loss in Weight Following Various Desiccation Periods			
	1 Hour	2 Hours	6 Hours	24 Hours
Check	+15.44	28.00	59.40	92.31
4 ppm.	18.30*	30.34	68.83**	94.32**
8 ppm.	17.25	31.42*	70.34**	94.42**
12 ppm.	19.90**	36.86**	76.65**	94.26**
L.S.D. at 5% level	2.75	3.27	4.06	0.96
L.S.D. at 1% level	3.95	4.70	5.83	1.39

+ Mean of 4 replicates desiccated at 27 degrees Centigrade
* Significant at the 5 percent level
** Significant at the 1 percent level

Twelve ppm. of TCA resulted in a significant increase, at the 1 percent level, in the amount of weight lost after a desiccation period of 1 hour or more when compared to the corresponding control. A similar increase, at the 1 percent level, was found in seedlings grown in 4 and 8 ppm. of TCA only after a desiccation period of 6 and 24 hours. At the end of 1 and 2 hours of desiccation the 4 and 8 ppm. treated seedlings had lost a greater amount of weight than the corresponding controls.

In all determinations, the seedlings treated with Dalapon or TCA lost a greater amount of weight than the corresponding untreated controls although the amounts were not always statistically significant.

The results of the biochemical analysis of the etiolated sugar beet seedlings grown in solutions of 0, 4, 8 and 12 ppm. of the growth substances Dalapon and TCA are given in Table 7.

Table 7

Analyses for Various Fractions Present in Etiolated Sugar Beet Seedlings

(Mean of 2 replicates)

A. Dalapon Treated Seedlings

Treatment	Percent Moisture	Percent Dry Matter	Percent Reducing Sugar	Percent Total Sugar	Percent Nitrogen	Percent Water Soluble Nitrogen	Sugar Per Gram of Dry Matter
Check	95.19	4.81	12.66	13.93	3.32	0.54	0.67
4 ppm.	94.97	5.03	14.31	15.31	3.30	0.58	0.77
8 ppm.	94.67	5.33	16.14	17.07	3.28	0.57	0.91
12 ppm.	94.63	5.37	17.13	17.69	3.36	0.58	0.95

B. TCA Treated Seedlings

Treatment	Percent Moisture	Percent Dry Matter	Percent Reducing Sugar	Percent Total Sugar	Percent Nitrogen	Percent Water Soluble Nitrogen	Sugar Per Gram of Dry Matter
Check	95.16	4.84	11.78	11.98	3.21	0.60	0.58
4 ppm.	94.91	5.09	13.95	14.73	3.02	0.57	0.75
8 ppm.	94.78	5.22	13.79	13.79	3.34	0.51	0.72
12 ppm.	94.57	5.43	15.28	15.65	3.28	0.57	0.85

The data presented indicate several trends. For example, the moisture content of the seedlings treated with either of the chemicals decreases when the concentration is increased from 0 to 12 ppm. and conversely the percent dry matter increases with increasing concentration of chemical used. Associated with the increase in dry matter is an increase in both the percent reducing and total sugars present. Apparently there was an increase in the sugar present per gram of dry matter.

Although the sugar content of the seedlings rose as a result of the chemical application, the nitrogen fractions as calculated on a dry weight bases showed no consistent differences or trends.

5. Discussion and Conclusions

From the data presented on the length of the roots and shoots of the etiolated seedlings, it is apparent that 12 ppm. of Dalapon and TCA inhibited the sugar beet seedlings more than the other treatments. Although the inhibition was significant, the 12 ppm. treatments were included in the various experiments in order that any differences that were present might be magnified.

The results obtained by visual observations on survival after low temperature treatment of seedlings previously grown in Dalapon and TCA solutions are similar to those obtained by Corns (8, 11). With respect to the amount of exosmosis in herbaceous material exposed to freezing temperatures, several workers have reported success in correlating the amount of diffusion with the cold resistance of the plant (13, 15). As mentioned earlier, certain limitations must be considered in order to evaluate the results presented. Differences between replicates were very pronounced even when strictest adherence to the procedure outlined was followed. It is felt that more reliable results could be obtained by use of a larger sample and greater replication.

Another of the difficulties encountered with the "exosmosis" procedure is associated with the choice of a time

interval between freezing and the determination of the amount of electrolytes that have diffused out of the plant. In the results obtained in the laboratory trials, the amount of injury after exposure to -10 degrees Centigrade for a period of 5 minutes varied from approximately 20 percent, after an exosmosis period of 5 hours, to over 100 percent if the seedlings were left in the solutions for 96 hours or more. For this reason an arbitrary time interval of 24 hours in the water bath was chosen for uniformity in the tests.

In spite of the above mentioned difficulties it would appear that the extent of injury as determined by the amount of diffusion of electrolytes from the seedlings, following low temperature exposure, was less for seedlings treated with Dalapon and TCA than for the corresponding untreated controls.

As reported earlier, no reference has been found in which Dalapon or TCA have been reported to increase the rate of desiccation from detached sugar beet seedlings. It is clear that treatment of sugar beet seedlings with both Dalapon and TCA has resulted in an increased rate of loss in weight when the severed seedlings are desiccated at room temperature and compared with the untreated controls. It should be noted that the differences between replicates were not significant in any of the experiments reported here. It is assumed that the amount of weight lost after the various desiccation periods is due mostly to water loss although respiration losses may be important components.

Mitchell and Marth (24) found that dipping severed shoots of bean plants into various concentrations of growth substances reduced the rate of water loss when compared to the untreated plants which were also dried at room temperatures. The differences between the two apparently contradictory results may be explained on the bases of concentration. Mitchell and Marth used concentrations 10 to 25 times higher than those reported in this investigation. It is also possible, however, that the differences may be due to the different reactions of the growth substances.

The significance of the results, relating the greater rate of weight loss of severed etiolated sugar beet seedlings treated with Dalapon and TCA, as an explanation for the apparent increased resistance to low temperature exposure is not too clear. It might be expected that the treated seedlings with their increased rate of apparent water loss should be able to withstand exposure to freezing temperatures with less injury than the controls. The question arises as to the importance of an increased rate of water loss in the type of quick freeze test used. The data do, however, support the view that with a slow rate of freezing an increased rate of water loss in the treated seedlings might be an important factor in increasing the resistance to low temperatures under practical conditions. Levitt (21) concluded that in closely related species an increased transpiration rate may be an important factor in frost hardiness. It should be pointed out that the present results reported do not refer to transpiration but rather to desiccation rate of severed sugar beet seedlings. The results

suggest that further work pertaining to transpiration and to respiration of the seedlings would be of great interest.

The results of the biochemical analyses of the etiolated sugar beet seedlings show that the chemically treated seedlings had a decreased moisture content and an increased dry matter content when compared to the untreated controls. The data presented in this thesis are somewhat similar to those obtained by Brown (3) who found an increase in dry matter in the basal regions of bean plants that were sprayed with 2,4-D.

Several workers (12, 29, 30, 32, 33) have reported an increased content of reducing sugars in plants after treatment with growth substances. From the data presented it can be seen that Dalapon and TCA have resulted in an increase in the amount of sugar present, with the increase being greatest in the reducing sugar content.

Levitt (21), after reviewing the work on the relationship of sugar content to hardiness in plants, concluded that within closely related varieties the resistance to low temperature exposure is usually positively correlated to the concentration of sugars present, if all other factors are equal. The increase in sugar noted would result in an increase in cell sap concentration and thereby lower the freezing point. Although in the present work the reported increase in sugar content is not very large, the increase may have some real bearing on differences obtained by the freezing tests.

It seems reasonable to conclude that the decreased moisture content associated with an apparently increased rate of water loss as well as a greater sugar content resulting in an increased cell sap concentration would, in part, explain the improved resistance to low temperature exposure in the chemically treated seedlings. It is, of course, more likely that the combination of factors rather than any one individual factor is responsible for such differences.

Obviously further work must be undertaken involving a slow freezing technique or conditions more nearly resembling those of the field. It would be of further interest to determine the effects of the growth substances on seedlings grown in the light with regard to resistance to low temperatures and to biochemical composition. As a step in the right direction, Corns (9) has reported on the low temperature resistance of treated seedlings grown under conditions of weak illumination. There definitely appears to be an important place for more intensive work of this nature.

SUMMARY

1. Dalapon and TCA applied at various rates and stages of development did not improve the low temperature resistance of sugar beets grown under field conditions at Edmonton during 1954 and 1955.

2. Dalapon and TCA applied as pre-emergent sprays at 8 pounds per acre reduced the root yield per acre significantly. The pre-emergent sprays of 2, 4 and 8 pounds of Dalapon as well as 4 and 8 pounds per acre of TCA lowered the yield of sucrose per acre, but

the reduction was not statistically significant when compared to the untreated controls.

3. The nitrogen content of the storage roots was increased significantly, at the 5 percent level, by pre-emergent applications of TCA at 4 and 8 pounds per acre.

4. Foliage applications of Dalapon and TCA, applied during 1955, increased the yield of sucrose per acre by as much as 10 percent when compared to the controls although the increase was not statistically significant. The increase in sucrose yield was manifested mainly through increased root yield, although in some cases the percent sucrose was higher than in the controls.

5. Rates of application, as high as 0.32 pounds per acre of Dalapon and TCA, applied to the foliage of sugar beets resulted in an apparently increased desiccation rate in detached leaves although the increased rate of loss was not significantly greater than the untreated checks.

6. Etiolated sugar beet seedlings treated with 4, 8 and 12 ppm. of Dalapon and TCA were more resistant to low temperature exposure than the corresponding controls. Two methods of evaluating injury were used and in both cases the shoots of the treated plants were more resistant than the non-treated material.

7. The etiolated seedlings, grown in solutions of 4, 8 and 12 ppm. of Dalapon and TCA, had a decreased moisture content when compared to the controls. Associated with the loss in moisture was an increase in sugar content, mainly reducing sugars. The increase in reducing sugars was in percentage as well as in the amount per gram of dry matter.

8. The increase in sugar content, presumably associated with an increased cell sap concentration and the increased desiccation rate of the treated seedlings are suggested as an explanation for the increased resistance to low temperature exposure in the treated sugar beet seedlings.

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